



Patent
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of) MAIL STOP AF
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Osamu SUZUKI et al.) Group Art Unit: 1651
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Application No.: 09/389,318) Examiner: I. Marx
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Filed: September 3, 1999) Confirmation No. 5287
)
For: METHOD FOR PRODUCING)
HIGHLY UNSATURATED FATTY)
ACIDS AND LIPID CONTAINING)
SAME)

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DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Kengo Akimoto, hereby declare as follows:

1. I am a co-inventor for the above-identified application.
2. The following experiment was performed either by me, or under my direct supervision and control.

3. Microorganisms Used for Experiments:

Three kinds of strains were used for this experiment. *Mortierella alpina* SAM 2197 and *Mortierella alpina* CBS608.70 were chosen as strains having resistance to high concentration of carbon source, and *Mortierella alpina* ATCC 42430 was chosen as a strain that does not have this property. The three *Mortierella alpina* strains used were thus:

Mortierella alpina SAM 2197 (strain which is described in instant specification and falls within scope of claimed subject matter; this strain belongs to the species *Mortierella alpina*);

Mortierella alpina CBS608.70 (strain which falls within scope of claimed invention, e.g., claims 1 and 33; this strain belongs to the species *Mortierella alpina*); and

Mortierella alpina ATCC 42430 (described in Kyle reference, U.S. Patent No. 5,658,767; this strain belongs to the species *Mortierella alpina*).

It is noted that *Mortierella alpina* CBS608.70 is stored at Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, and was known prior to the priority date of the present invention. However, it should be noted that its carbon source resistance was not known.

4. Materials and Methods:

Five liters of the following medium was prepared in a 10-liter jar fermentor:

Glucose 4%, yeast extract 2%, soybean oil 0.2%, adekanol 0.01%, pH 6.3.

The medium was sterilized at 120°C for 30 minutes. One hundred milliliters of a preculture solution of the strains was inoculated into the sterilized medium. Culturing with aeration and agitation was conducted for 9 days at 28°C at the aeration rate of 1 vvm with stirring at 300 rpm.

Glucose 2% (as concentration in culture broth) was added on the second, third and fourth day of culturing. After completion of the culture, the amount of arachidonic acid

produced were determined using the same method as an example of Suzuki et al (*see*, PCT application WO 98/39468, Example 1).

5. Results:

Mortierella alpina SAM 2197 and *Mortierella alpina* CBS608.70 have resistance to a carbon source of high concentration. Therefore, the amounts of arachidonic acid produced were higher than 7 g/L. However, *Mortierella alpina* ATCC 42430 does not have resistance to a carbon source of high concentration, and the amount of arachidonic acid produced was lower than 7 g/L.

Table 1. Results

Strain	Amount of arachidonic acid produced
<i>Mortierella alpina</i> SAM 2197	7.4 g/L
<i>Mortierella alpina</i> CBS608.70	7.2 g/L
<i>Mortierella alpina</i> ATCC 42430.	4.0 g/L

6. In this experiment, I compared the above-identified three strains for their arachidonic acid productivity under the same conditions, in accordance with claims 1 and 33, for example.

7. As can be seen from the results shown in Table 1, the strains *Mortierella alpina* SAM 2197 and *Mortierella alpina* CBS608.70, which fall within the scope of the instant claims, produced more than 7 g of arachidonic acid/L culture medium. By

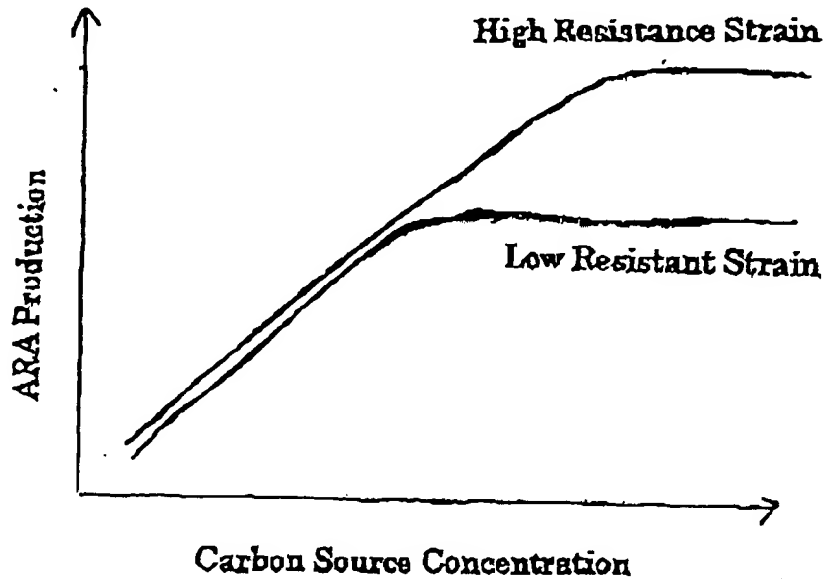
comparison, the strain *Mortierella alpina* ATCC 42430 of the Kyle reference produced only 4.0 g of arachidonic acid/L culture medium.

8. Theoretically, the maximum amount of cultured cells should increase as the total concentration of a carbon source in a culture medium increases. As a result, the total amount of product, i.e., arachidonic acid, should increase as the total concentration in culture medium.

9. However, in practice, a high concentration of carbon source inhibits the growth of the producer microorganism. As a result, the maximum level of product is limited. In the case where a microorganism having lower resistance to carbon source concentration is used, the growth of cells stops at a lower level of cell concentration, even if a culture medium contains a high level of carbon source concentration, due to inhibition by the carbon source. As a result, the maximum level of the product is low.

10. On the other hand, in the case where a microorganism having higher resistance to carbon source concentration is used, the growth of cells stops at a higher level of cell concentration, if a medium contains a high level of carbon source concentration. As a result, the maximum level of the product is high.

11. Schematically, the above explanation can be expressed as follows:



12. I further declare that I am aware that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and my jeopardize the validity of any patent application or any patent issuing thereon. All statements made of my own knowledge are true, and all statements made on information and belief are believed to be true.

August 7, 2003
Date

Kengo Akimoto
Kengo Akimoto, Ph.D.